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TECHNICAL MANUSCRIPT 226

STAPHYLOCOCCAL ENTEROTOXEMIA: PATHOLOGIC LESIONS IN RHESUS MONKEYS EXPOSED BY AEROSOL

Peter J. Soto, Jr.

William G. Roessler

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Peter J. Soto, Jr.

William G. Roessler

Pathology Division
DIRECTORATE OF MEDICAL RESEARCH
and
Medical Investigation Division
DIRECTORATE OF BIOLOGICAL RESEARCH

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In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

Thirty rhesus monkeys were given purified staphylococcal enterotoxin B aerogenically. A method for calculating the dosage is referenced. Twenty-two animals responded with emesis and/or diarrhea within 5 hours after exposure, 9 died spontaneously, and 21 were sacrificed sequentially up to 7 days after exposure. The only pathologic lesions attributable to the challenge were severe pulmonary edema (with resolving fibrinous exudate in one animal sacrificed at 7 days), edematous enlargement of the tracheobronchial lymph nodes, and vacuolar nephropathy, presumably of hypokalemic origin, in one instance. Alveolar capillary block from pulmonary edema seemed the most important cause of death in nine animals. Eight challenged and two control animals remained symptom-free and showed none of the above lesions postmortem. Other possible mechanisms of death are discussed. The absence of significant lesions in the gastrointestinal tract strongly suggests that enterotoxemia occurred, and that emesis and diarrhea may have been caused by toxic injury to appropriate areas in the medulla and pons.

I. INTRODUCTION

Enteritis resulting from the ingestion or injection of staphylococcal enterotoxin had been studied in man[1-4] and in experimental animals.[5-14] This report concerns the clinical picture and pathologic alterations in monkeys exposed to a highly purified enterotoxin by aerosol inhalation. This route has not, to our knowledge, been used by other investigators.

II. MATERIALS AND METHODS

Thirty-two clinically well, tuberculin-negative, young adult rhesus monkeys of mixed sex with a mean body weight of 2.93 kg and (standard deviation ± 0.46 kg) were housed two per cage for 12 to 14 weeks prior to use. During the holding period they were fed commercial monkey chow and given water ad libitum. Daily observation showed no diarrhea or other manifestations of illness.

Thirty monkeys were exposed for 4 minutes to aerosols of a highly purified preparation of enterotoxin B[15] in a modified Henderson apparatus enclosed in a ventilated cabinet system according to the methods of Roessler and Kautter.[16] The toxin was purified from culture filtrates of Staphylococcus aureus, strain S-6, by Dr. E.J. Schantz, using a modification[17] of the procedure described by Bergdoll. The toxin was at least 99.5% pure and contained no detectable lysins of any type.

A dye tracer technique was used to estimate the concentration of enterotoxin in the aerosol. Uramine (fluorescein sodium) at a concentration of 20 µg per cc was incorporated in the enterotoxin solution and aerosolized by the Collison spray device. The cloud was sampled continuously during the 4-minute exposure of the animals, using short-stemmed all-glass impingers containing water. Since the dye and the enterotoxin are water-soluble, the concentration of the dye in the impinger as measured in a fluorometer* is indirectly a measure of the toxin concentration. Using a dilution of the spray solution as a standard, and knowing the rate of aerosol flow through the impinger, doses for the exposed monkeys can be estimated. To determine the dose per kg of body weight, the method described by Guyton[18] was used. The calculated inhaled dose range of enterotoxin administered to the monkeys varied from 17.0 to 59.6 µg per kg with a mean of 34.7 µg per kg.

* Photovolt Corporation, New York.

Following exposure, the monkeys were maintained in open wire cages and observed continuously for clinical responses for 5 hours following exposure. Thereafter, all animals were observed four times daily, particularly for diarrhea, vomiting, depression, and death, throughout the entire observational period.

The remaining two animals were not challenged with enterotoxin but were placed in the same room with the exposed monkeys to serve as environmental room-controls.

Twenty animals were sacrificed in random groups of four at 12, 24, 48, 72, and 96 hours after exposure by intracardiac injections of pentobarbital sodium, supplemented on occasions with an intravenous injection. One animal was sacrificed 7 days after challenge and the two room-controls on the 8th day. The remaining nine animals died between 48 and 72 hours after exposure.

Complete necropsies were performed immediately after euthanasia or as soon as possible after post-exposure death. At necropsy, total lung weights were determined, and the presence or absence of pleural effusion was recorded (Table 1). The tissues were fixed in buffered formalin and stained routinely with hematoxylin and eosin. Occasionally acid-fast, Gomori, periodic acid-Schiff (PAS), and Sudan IV stains were also employed.

III. RESULTS

A. CLINICAL OBSERVATIONS

As indicated in Table 1, 19 of the 30 challenged animals responded with emesis, 7 with diarrhea, and 4 manifested both of these signs. Eight of the challenged animals failed to respond clinically. The two control animals also remained symptom-free.

The duration of diarrhea or emesis did not exceed 12 hours, and in most instances recovery was complete within 5 hours. The average number of episodes of emesis or diarrhea was two and one respectively.

The period of time between exposure to enterotoxin and the onset of clinical response varied from 68 to 260 minutes, with an average of 150 minutes.

Nine animals died, and of these the exact time of death was recorded for animals numbered 14 through 17. The remainder were found dead on the morning of the second and third days after exposure. These animals were moribund when last observed on the previous evening; consequently, they could have been dead for a maximum of 11 hours (Table 1).

TABLE 1. EFFECTS OF ENTEROTOXIN B AEROSOL ON RHESUS MONKEYS[a]

Monkey Number	Clinical Response[b] Emesis	Diarrhea	Sacrificed(S) or Died(D), hours after exposure	Pulmonary Edema[b]	Pleural Effusion
1	X	-	12 S	-	-
2	X	-	12 S	-	-
3	-	-	12 S	-	-
4	X	-	12 S	-	-
5	-	-	24 S	-	-
6	-	-	24 S	-	-
7	-	X	24 S	-	-
8	-	X	24 S	-	-
9	X	X	48 D[c]	+++	3 cc
10	-	-	48 S	-	-
11	X	-	48 S	++	7 cc
12	X	-	48 S	-	-
13	-	-	48 S	-	-
14	-	-	53 D	+++	4 cc
15	-	-	53 D	+++	5 cc
16	X	-	58 D	+++	10 cc
17	X	-	60 D	+++	20 cc
18	X	X	72 D[c]	+++	-
19	X	-	72 D[c]	+++	3 cc
20	X	X	72 D[c]	+++	16 cc
21	-	X	72 D[c]	+++	-
22	X	-	72 S	+	-
23	X	-	72 S	+++	-
24	X	-	72 S	+++	-
25	X	-	72 S	+++	-
26	X	X	96 S	+++	-
27	X	-	96 S	+	-
28	-	-	96 S	+++	-
29	X	-	96 S	+++	-
30	X	-	168 S	+++	-
31[d]	-	-	192 S	-	-
32[d]	-	-	192 S	-	-

a. Exposure time 4 minutes.
b. X, Present; -, absent; +, minimal; ++, moderate; +++, marked.
c. Found dead.
d. Control.

B. PATHOLOGIC ANATOMY

1. Gross Observations

The principal gross morphologic changes were found in the thoracic cavity. When pulmonary edema was present the lungs were quite heavy, firm, deep red in color, and the cut surface exuded copious, pink, frothy fluid. The tracheobronchial tree contained fluid of a similar character. The ratio of lung weight to body weight in the 19 animals with edematous lungs was 20.3 gm per kg with a standard deviation (S.D.) of ±3.5 compared with 9.9 gm per kg (S.D. ±1.9) in the experimental monkeys without edema and 7.8 gm per kg (S.D. ±3.2) in sacrificed normal monkeys.[19] The hilar lymph nodes were edematous and enlarged. Pleural effusion was seen in eight animals, mainly in those with marked pulmonary edema. The fluid was clear, straw-colored, and contained coagulated masses of fibrin. The volume varied from 3 to 20 cc.

The gastrointestinal tract showed only infestation of the colon with the larvae of Oesophagostomum spp. The mucosa was clean and did not exhibit any evidence of sloughing.

The remaining organs were not remarkable except for enlargement of the adrenal glands in most instances and edema of the mesenteric lymph nodes.

2. Microscopic Observations

a. Lungs

The most significant and consistent morphologic change observed was the presence of pulmonary edema. The fluid was eosinophilic and rich in fibrin. Peribronchial and perivascular edema was marked.

Edema was found in animals studied at 48 hours, and in most of those necropsied thereafter (Table 1). In the animals that lived less than 96 hours, the edema fluid was relatively cell-free and homogeneous (Fig. 1). In animals sacrificed at or after 96 hours, many plump macrophages containing pale PAS-positive material were noted (Fig. 2). Fat stains were negative.

In the monkey sacrificed at 7 days, a similar fluid was present, but many alveolar ducts and air sacs contained a fibrinous precipitate (Fig. 3) often associated with the formation of syncytial giant cells (Fig. 4).

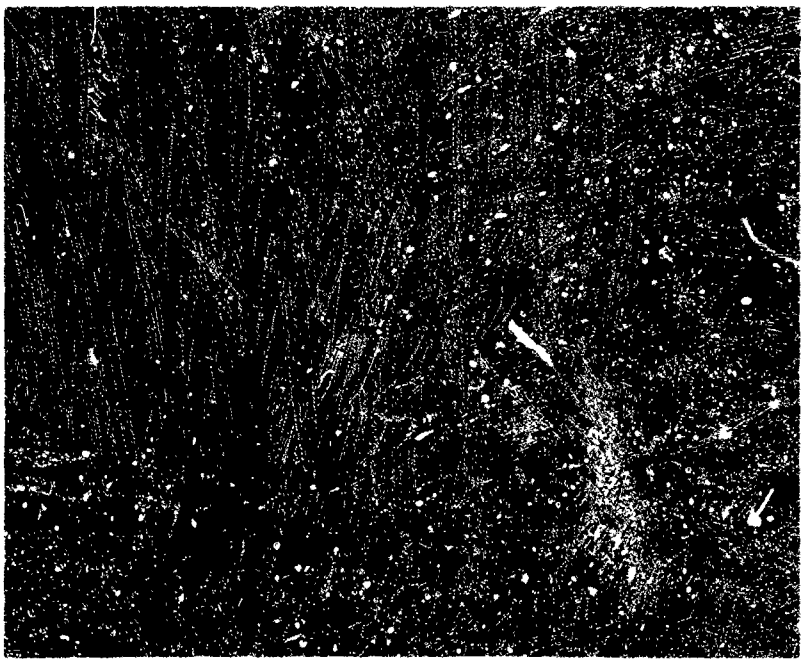


Figure 1. Typical Field of Lung Tissue Showing Marked Pulmonary Edema in the only 48-Hour Death. Stained with hematoxylin and eosin. 90X

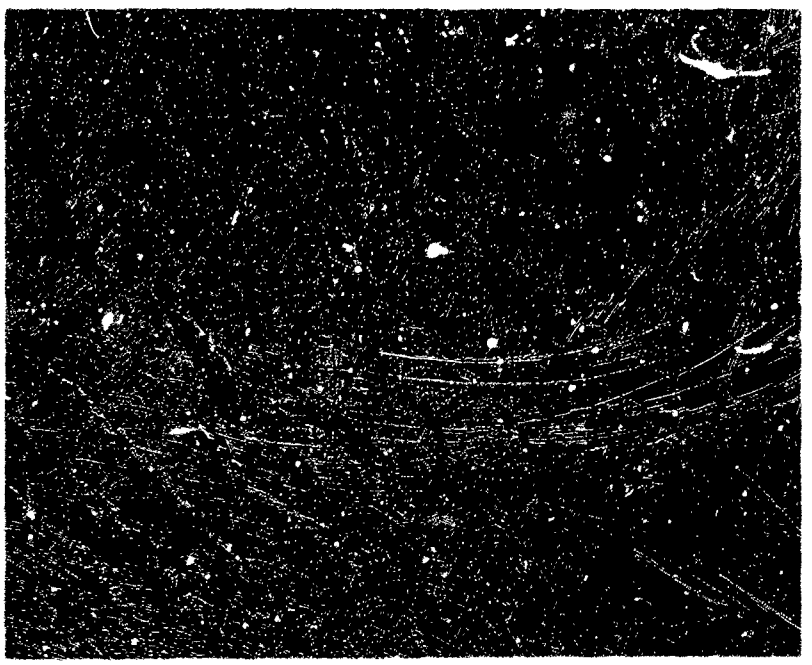
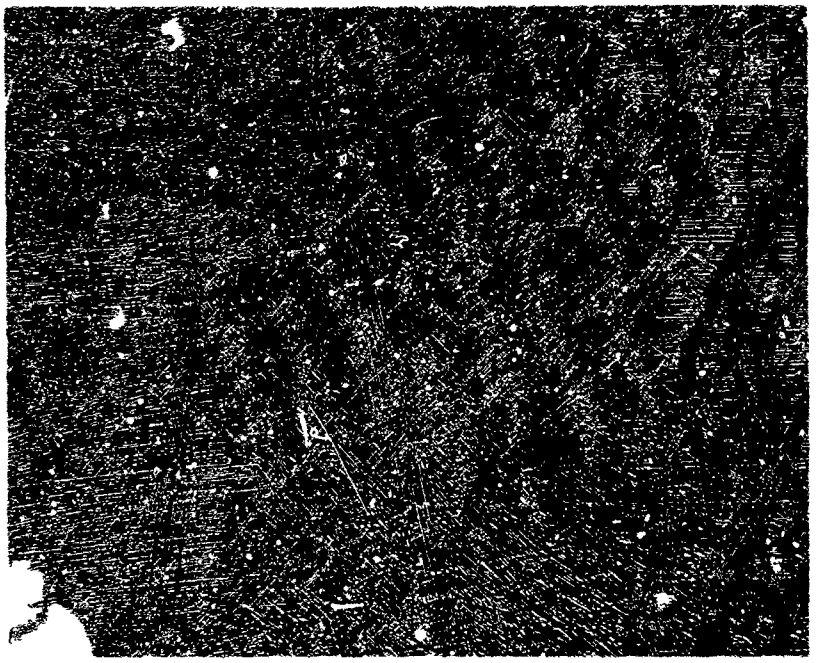


Figure 2. Edema Fluid in Lung Tissue Containing Numerous Plump Macropheges with Vacuolated Cytoplasm in a 96-Hour Sacrifice. Hematoxylin and eosin. 320X



e 3. Resolution of the Pulmonary Edema in Lung Tissue is Well-Established by 7 Days. Hematoxylin and eosin. 90X

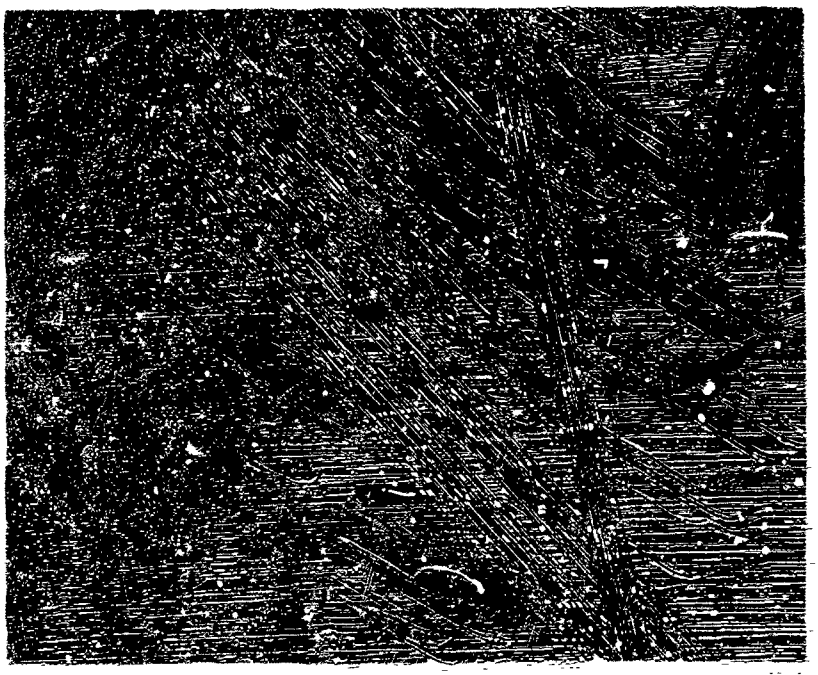


Figure 4. Lung Tissue with Higher Magnification Demonstrating Syncytial Giant Cells. Hematoxylin and eosin. 320X

b. Gastrointestinal Tract

The changes noted were identical in both challenged and control animals and consisted of moderate to heavy infiltration of the mucosa and submucosa of the stomach, duodenum, ileum, and colon with lymphocytes and plasma cells. The nidi of infestation of the colon with Oesophagostomum were characterized by submucosal areas of hemorrhage and necrosis rimmed by a collar of lymphocytes and giant cells.

c. Other Related Lesions

The lymph nodes, especially those of the tracheobronchial group, showed edema and marked hyperplasia of the lymphocytic elements. These responses were seen in most animals, including one of the controls.

The kidneys of 11 of the animals, including the two controls, showed varying degrees of hydropic degeneration of the convoluted tubules. For the most part the changes were seen in the superficial cortical tubules. One monkey (number 17) showed vacuolar nephropathy compatible with hypokalemia (Fig. 5). This same animal showed acute tubular necrosis, as did monkey number 11.

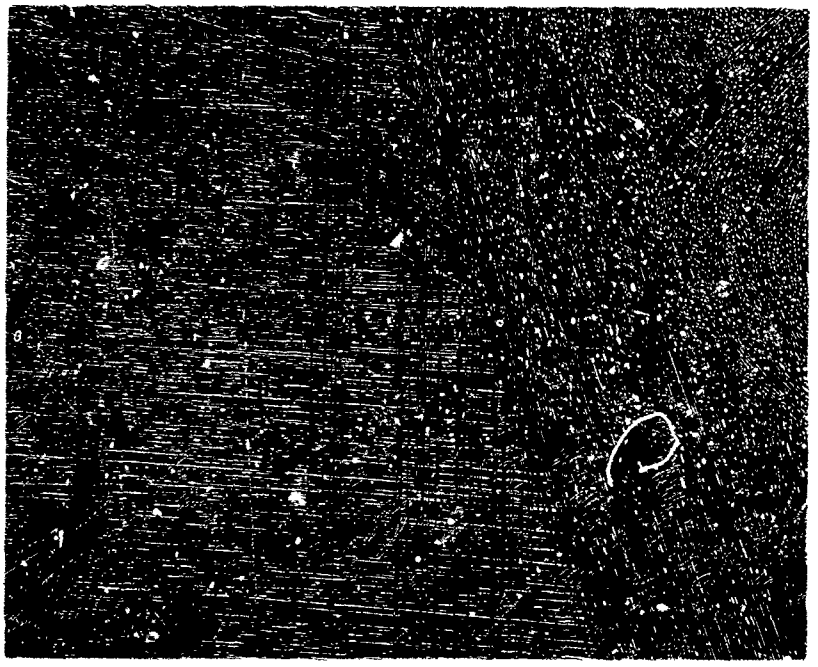


Figure 5. Kidney Tissue Showing Vacuolar Nephropathy of Proximal Convoluted Tubules in the 60-Hour Death. Hematoxylin and eosin. 320X

d. Lesions not Related to Enterotoxin

Lesions considered on the basis of previous observations[19] to be unrelated to the experimental challenge were "idiopathic" myocarditis, the formation of multinucleated hepatic parenchymal cells, infiltration of the gastrointestinal tract with lymphocytes and plasma cells, and infestation with nematodes, adrenal cortical hyperplasia, and salivary gland virus inclusion bodies in the kidneys. A perirenal granuloma was noted in monkey number 8 and a periadrenal abscess in monkey number 13. Both of these latter lesions are of unknown etiology.

The enlarged adrenals seen grossly were reflected by hyperplasia, principally of the zona fasciculata.

Morphologic changes in the central nervous system were meager and confined to the lenticular nuclei in three animals (numbers 24, 28, and 32). These changes consisted of focal collections of oligodendroglial cells and other round cells. Sections of the pons and medulla were not remarkable.

IV. DISCUSSION

This experiment was undertaken to determine the morphologic responses of animals to aerogenically introduced staphylococcal enterotoxin; to our knowledge, this had not been previously described.

Previous studies have been principally concerned with the physiologic aspects of enterotoxemia with little in the way of descriptive morphology except for the work of Prohaska[9] and Warren.[10] Both produced fatal enterocolitis in chinchillas by the oral administration of enterotoxin. In the latter experiment the degree of purity of the enterotoxin preparation varied considerably and all preparations produced enterocolitis. More recently, Warren[14] has produced acute enteritis as well as lesions believed to resemble regional enteritis in the dog, using the Maydl enterostomy.

Fatal human cases of staphylococcal food poisoning are rare and the available reports with complete pathologic examination showed different patterns. Weed's[2] cases exhibited pulmonary edema with focal alveolar hemorrhages. The gastrointestinal tract was not mentioned. The report of Dorlin[3] was apparently confined to a gross examination of the gastrointestinal tract, where evidence of inflammation was found. The case reported by Blackman[4] most probably originated as a case of food poisoning, but the autopsy findings were more consistent with pseudomembranous enterocolitis.

Palmer,[20] using gastroscopic biopsies, followed the morphologic changes secondary to intoxication with staphylococcal enterotoxin. The histopathologic changes were transient in that necrosis was confined to the superficial portions of the mucosa and restoration was complete in 92 hours.

Some animals in the experiment described here apparently tolerated the presence of pulmonary edema despite the significant degree of involvement. This impression is based on the observation that the sacrificed animals, although subdued, did not show evidence of respiratory embarrassment even immediately prior to the time of the sacrifice. One can only speculate how long these animals would have tolerated the presence of the edema. On the other hand, all the animals that died were moribund for several hours before death, and while moribund they appeared to experience some respiratory distress. In these animals possible mechanisms of death include alveolar capillary block from the pulmonary edema, electrolyte imbalance, and a direct toxic injury to the respiratory center.

The mechanism by which enterotoxin produces pulmonary edema is not completely known but is most probably mediated through the vagus nerves.[18] The sequence of events may be that the vagal nuclei emit impulses that result in altered permeability of the pulmonary capillaries, permitting escape of protein-rich fluid into the air sacs. This concept is supported to a certain extent by the fact that the emetic effect of enterotoxin can be prevented by ablation of the medullary area that contains the vagal nuclei.

The cause of pulmonary edema in monkeys dying after inhalation of enterotoxin is not known. Pulmonary edema has also been observed in monkeys dying as a result of intravenous injections of comparable doses.*

The lack of specific morphologic changes in the gastrointestinal tract, even in those animals with emesis and/or diarrhea, is difficult to explain. It may be that the monkey, in contrast to other animals, responds clinically but not pathologically. Furthermore, the fact that eight of the animals failed to respond to the enterotoxin is interesting and equally puzzling. This phenomenon has been observed in previous studies in a certain percentage of monkeys, regardless of the route of administration. We have observed that at the doses administered in this experiment emesis and/or diarrhea occurred in 75 to 85% of the monkeys.* Most probably this is not related to dose, as several of the animals without emesis or diarrhea received doses in the same range as those that responded. It may be that some monkeys are genetically resistant and consequently do not respond regardless of the dose. Other factors such as acquired immunity to enterotoxin, physical condition, and fluid balance may also play a role in determining the presence or absence of a clinical response.

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* Sato, P.J., Jr. Unpublished data.

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